

**ANTERIOR PART OF THE EYE AS A MODEL FOR THE
DEVELOPMENT OF INTRAOCULAR PRESSURE MEASUREMENT
DEVICE**

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Glaukooma on näköhermon etenevä sairaus, joka aiheuttaa vaurioita papillaan, hermosäiekerrokseen ja näkökenttään. Glaukooman tärkein hoidettavissa oleva riskitekijä on koholla oleva silmänpaine. Silmänpaine voidaan mitata eri menetelmiin perustuvien tonometrien avulla silmän ulkopuolelta. Tonometrien antamat lukemat ovat alttiita eri virhelähteille, joista keskeisin on sarveiskalvon paksuus. Tässä työssä vertailtiin neljää eri tonometriä (Icare, Pneumatonometri, Tono-Pen XL ja Goldmannin aplanaatiotonometri) silmän sisältä mitattuun todelliseen painelukemaan sian ja lehmän silmillä.

Työssä sian ja lehmän silmät kiinnitettiin muoviseen painekammioon, jonka painetta muutettiin 5 ja 50 elohopeamillimetrin (mmHg) välillä nestepatsaan korkeutta muuttamalla. Silmänpainemittareiden antamat lukemat kirjattiin 5 mmHg:n välein. Kaikki neljä tonometriä antoivat johdonmukaisesti todellista painelukemaa alhaisempia lukemia yli 10 mmHg:n painetasoilla sekä sian että lehmän silmillä.

Todennäköisesti tonometreilla saadut alhaisemmat painelukemat johtuivat siitä, että tutkittavat silmät olivat huomattavan ödemaattisia. Sian silmien sarveiskalvon paksuus oli heti enukleaation jälkeen mitattuna keskimäärin 870 mikrometriä kun myöhemmin silmänpaineen mittaushetkellä mitattuna se oli 6 silmästä yli 1000 mikrometriä ja lopuista neljästä silmästä 961 mikrometriä. Lehmän silmät olivat enukleaation jälkeen mitattuna yli 1000 mikrometriä. Jatkossa sarveiskalvon ödeemaa voitaneen vähentää säilömällä silmiä hypertonisessa liuoksessa ennen mittaushetkeä. Lisäksi sian ja lehmän silmät ovat kaarevuussäteeltään ihmissilmää suurempia, mikä kirjallisuuden mukaan saattaa vaikuttaa mittaustuloksesta alentavasti ainakin Goldmannin aplanaatiotonometriin.

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1 INTRODUCTION

Glaucoma, a progressive optic neuropathy, is the second leading cause of blindness worldwide (1) and its prevalence is estimated at 60.5 million in 2010 with over 8 million people bilaterally blind from the disease. The most common forms of glaucoma are the primary angle-closure glaucoma (PACG) and the primary open-angle glaucoma (POAG), which differ in terms of whether the angle between the iris and cornea is open or closed. POAG, which accounts for approximately 75% of the total glaucoma, develops at a slow pace and without initial symptoms. (2) It typically has an adult onset and a great proportion of patients are unaware of the condition until the later stages(3). The slow apoptotic death of retinal ganglion cells and their axons in POAG causes the characteristic changes in the optic nerve head and the retinal nerve fiber layer and the loss of visual field(4). Early detection and treatment is important, because the blindness caused by glaucoma is irreversible.

An elevated intraocular pressure is considered the most significant risk factor for the development of glaucoma(5,6). Intraocular pressure (IOP), which is considered to be in a statistically normal range between 10 and 21 mmHg, describes the balance between the production and outflow of the aqueous humor. The aqueous humor is secreted from the ciliary body in the posterior chamber, from where it flows through the pupil to the anterior chamber. Most of the outflow is through the trabecular meshwork, Schlemm's canal and to the venous circulation. The remaining fluid exits through the uveoscleral routes.(7)

Although the exact mechanism is not known, a certain level of IOP depending on the eye's pressure-susceptibility is thought to cause the compression of ganglion cell axons and to lead to the blockade of axonal transport and insufficiency of trophic factors for the ganglion cells. A decreased blood flow to the optic nerve may also contribute to the glaucomatous damage either independently or in conjunction with the IOP. Other factors, such as glutamatergic stimulation, may also play a role in the pathophysiology of glaucoma.(7)

Although approximately one third of the people with POAG have their IOP in the statistically normal range and are classified as having normal-tension glaucoma(8), the risk for glaucomatous damage increases with increasing IOP. For example, if the IOP is 22-29 mmHg or over 30-35 mmHg the

relative risk is 13 or 40, respectively.(5) Other risk factors for developing glaucoma include older age(9), a positive family history of glaucoma(10,11), high myopia(12), exfoliation(13) and black race(14).

Reducing intraocular pressure by medication, laser or surgery is currently the basis of glaucoma treatment and the only treatable risk factor. The IOP reduction treatment has been reported effective in preventing or delaying glaucoma development and progression in randomized clinical trials. In the Ocular Hypertension Treatment Study (OHTS)(15) with patients having elevated IOP but no signs of glaucomatous damage at baseline, progression occurred in 4.4% and 9.5% of the medical therapy and control groups, respectively, in the five-year follow-up period. In the Early Manifest Glaucoma Trial (EMGT) the progression of newly detected POAG was less frequent in the surgical treatment group (progression observed in 42% of the patients) than in the control group (62%) during the six-year follow-up period. Progression occurred on average 18 months later in the treatment than the control group.(16). The findings of EMGT and OHTS were similar to the findings of the Advanced Glaucoma Intervention Study, where all patients underwent glaucoma surgery at baseline. Lower achieved IOP during post-operative six-year follow-up period was associated with reduced progression of visual field damage. (17) The Collaborative Initial Glaucoma Treatment Study compared the effectiveness of different treatment methods. After at least four years of follow-up, no significant difference in outcome was found between patients initially treated with medication or surgery despite the fact that IOP was on average 3.5 mm Hg lower in the group that received surgical treatment.(18)

Effectiveness of IOP reduction in normal tension glaucoma was studied in the Collaborative Normal Tension Glaucoma Study(19), where patients were randomized to treatment and control groups. When the control group was followed up from a baseline at the time of randomization and the treatment group from a new baseline after stable IOP reduction had been established, a slower rate of glaucomatous progression for the treatment than the control group was reported. In another analysis where both groups were followed up from the same baseline at the time of randomization, no difference in glaucoma progression between treatment and study groups was found. However, IOP reduction had a beneficial effect if cataract cases, which were more common among those who received treatment, were excluded from the data.(20)

Due to the aforementioned facts, the measurement of IOP has a fundamental role in the risk assessment of glaucoma and in monitoring the effectiveness of IOP reduction treatment. IOP measurement, nevertheless, is not suitable for screening purposes because relying merely on the IOP in glaucoma diagnostics results both in false negative and false positive results; some glaucoma patients have normal tension glaucoma and some individuals have elevated IOP without glaucoma, a condition that is called ocular hypertension.

The IOP can be measured either non-invasively by means of tonometry or invasively from the intraocular space. All tonometers, however, have problems with accuracy because the measurement is indirect and affected by the limiting membrane between the tonometer tip and the intraocular fluid. For instance, if the IOP is measured from the surface of the cornea, the most obvious factor affecting the accuracy is the central corneal thickness, which varies among individuals. There are several commercially available tonometers with varying degrees of accuracy and dependence on corneal biomechanical properties. However, even tonometers with good accuracy do not necessarily reflect person's true IOP because the currently available tonometers are capable of measuring IOP at single time points only.

Recently, intraocular pressure fluctuation has aroused interest as a possible independent risk factor for glaucoma. IOP fluctuates in a 24-hour pattern being highest during the nocturnal period both in healthy subjects and in glaucoma patients (21)(22). It seems that the fluctuation is greater for those eyes that have higher mean IOP. Consistently, the fluctuation is reported to be higher in individuals with glaucoma than in healthy subjects(23). However, the role of either 24-hour IOP fluctuation or long-term fluctuation in IOP between office visits as a risk factor in glaucoma has yet to be clarified. While IOP fluctuation has been found an independent risk factor for glaucoma in some studies, other studies have concluded the opposite(24). The uncertainty in these studies arises from the fact that, due to the lack of a continuous IOP measurement device, the fluctuation curves are extrapolated from IOP measurements at single time points. The subjects have also to be awakened during the night time measurements, which might influence the physiological IOP fluctuation pattern.(21) The continuous measurement of IOP would therefore allow a better monitoring of glaucoma patients, a more precise adjustment of IOP reduction treatment and a more comprehensible understanding of the normal IOP fluctuation pattern.

The aim of this study is to evaluate the feasibility of an experimental *in vitro* intraocular pressure model with porcine and bovine eyes for the development of an intracorneally implantable continuous intraocular pressure monitoring device. In the model, the pressure inside the eye is adjusted manometrically to different pressure levels followed by the measurement of the IOP outside the eye by means of four commercially available tonometers. The results of manometry and tonometry are then compared to decide whether the tonometers can with a sufficient accuracy track the changes in IOP in this experimental model.

2 REVIEW OF LITERATURE

As this study uses the Goldmann Applanation tonometer (GAT), pneumatonometer, Tono-Pen XL and Icare to evaluate the experimental intraocular pressure model, the literature was reviewed with regard to the accuracies of these tonometers.

2.1 *The Goldmann Applanation Tonometer*

Applanation tonometry can be affected by many sources of error among of which the central corneal thickness (CCT) is the most obvious. Generally, IOP is overestimated in structurally thick corneas and underestimated in thin corneas. (25) In a major review on the correlation of CCT and applanation tonometry, authors reported a 1.1 mmHg increase in tonometry for a 10% increase in CCT with normal eyes and using linear regression model. For eyes with chronic disease, such as POAG, ocular hypertension or normal-tension glaucoma, the increase was 2.5 mmHg. (26) Liu et al, however, suggested in their report that the corneal biomechanical properties other than CCT may play a more prominent role in tonometry measurements (27). A small corneal radius of curvature (i.e. steep cornea) has also been reported to overestimate and corneal edema to underestimate IOP with applanation tonometry. The underestimation of IOP in thick but edematous cornea might be due to the fact that it is easier to applanate than a non-edematous cornea. (25)

2.2 The Pneumatonometer

A positive correlation between pneumatometry and CCT has been found in some studies(28)(29), while some studies have not reported a correlation.(30)(31). Compared to GAT, Condon et al reported that results did not differ in glaucomatous eyes but that pneumatometry overestimated IOP in normal eyes by 1-3 mmHg(32). Bayraktar et al, too, reported no difference between the methods in the eyes of glaucoma suspects(31). In a study by Singh et al no statistical difference was found between the two methods in normal or hypertensive eyes or eyes with primary OAG or normal-tension glaucoma, even though the difference was greater in hypertensive eyes and eyes with primary OAG(28).

2.3 The Tono-Pen XL

Several studies have reported a positive correlation between CCT and Tono-Pen IOP. (33)(34)(35)(36)(37) In three most recent studies found, the reported increase in Tono-Pen IOP has been 2.2 mmHg(35), 2.1 mmHg(36) and 3.1 mmHg(37) for 100 microm increase in CCT. Compared to GAT, some studies have reported Tono-Pen to underestimate GAT readings(38)(39) while some have reported it to underestimate GAT especially at high IOP (40)(41). At low IOP Tono-Pen either shows no statistically significant difference(42) or overestimates IOP(41)(43) when compared to GAT. In addition, some studies have reported no significant difference between Tono-Pen and GAT readings(44)(45). Two studies that investigated the effect of obtaining IOP from central and mid-peripheral cornea found that these two results did not significantly differ. (33)(45) Corneal radius of curvature has been reported to have no significant effect on Tono-Pen measurements in one study (35).

2.4 The Icare Rebound Tonometer

CCT seems to affect also rebound tonometer measurements. One study found no correlation between CCT and Icare in multiple regression analyses when other corneal biomechanical factors were considered at the same time. Authors suggested that corneal factors other than CCT influence Icare measurements more than thickness alone. (46) However, in studies that did not consider these other corneal factors a positive correlation between CCT and Icare was found. (35,47-50) In three different recently published studies the IOP increase was 3.5 mmHg(35), 7.0 mmHg(50) and 8.0 mmHg(49) for

100 microm increase in CCT. A study that investigated the effect of corneal radius of curvature on Icare measurements found no significant correlation.(35) Icare accuracy has been compared to other tonometers, mainly to Goldmann applanation tonometer. A good correlation between Icare and GAT is reported in several studies (48) (51) (49) (52) (53) (54) (55). Although the correlation is good, Icare has been found to overestimate IOP from 0.4 to 3.6 mmHg when compared to GAT (56) (49) (53) (47) (57) and the difference was reported to increase in higher IOPs in one study(52). A tonography effect of 0.6 mmHg in three successive measurement series was observed in one study (58). Measurements performed on nasal and temporal regions of cornea correlated well with central corneal measurements, even though they were reported to be slightly less than those obtained from the central cornea (59). In animal studies, Icare correlated well with manometric readings (60) (61) but underestimated modified GAT (61) and Tono-Pen XL values (62).

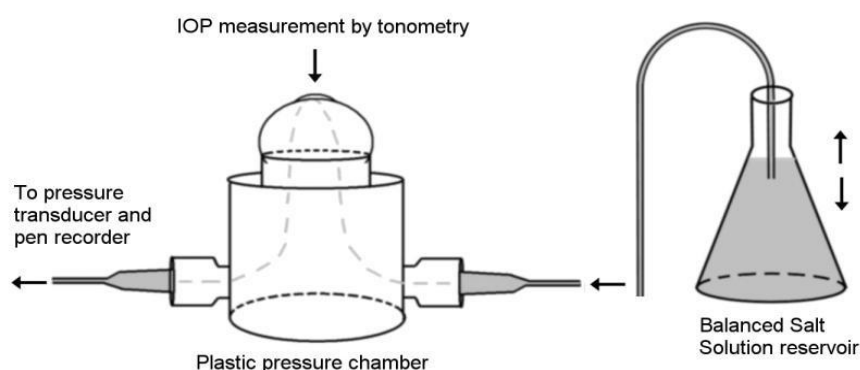
3 MATERIALS AND METHODS

The enucleated porcine and bovine eyes used in this study were provided by two local slaughterhouses. Eyes were excised immediately after slaughtering and prior to exposing the animals to steam treatment to maintain corneal integrity. Eyes were delivered to the measurement site in cooled containers. Those eyes that were not used in the same day were kept refrigerated and new eyes were ordered every week. Before measurements, the central corneal thickness of each eye was measured by means of an ultrasonic pachymeter (PachPen® Model 24-5100, Accutome Inc.) and the average of nine individual measurements displayed by the device was used for analysis. PachPen has a range of 300 to 999 micrometers. For those corneas that were beyond the measuring range of the device, a value of > 1000 micrometers was used for analysis.

An approximately two and three centimeter diameter area was removed from the posterior part of the porcine and bovine eye ball, respectively, through which the vitreous humor, lens and lens capsule were removed to gain access to the anterior and posterior chambers. Subsequently, the eye was mounted on to a plastic eye chamber and secured into position with a surgical suture material. Eye chambers of 22 mm and 32 mm outer diameter were used for porcine and bovine eyes, respectively.

The eye and the chamber together form a closed space, which can be filled with fluid to pressurize the eye (Figure 1). Two polyethylene tubes of 1.14 mm inner and 1.57 mm outer diameter (PE160 Polyethylene Tubing, Becton Dickinson and Company) were connected to the eye chamber, one of which allowed fluid to enter the system and the other of which allowed the fluid to leave the system. After fixation of the eye to the chamber, the system was filled with Balanced Salt Solution® (BSS) to test for possible leaks and to remove air from the system.

Figure 1 An experimental in vitro intraocular pressure model with porcine and bovine eyes. The pressure inside the plastic pressure chamber is adjusted by changing the height of the Balanced Salt Solution reservoir and monitoring the pressure from the pen recorder. Tonometry is performed at different pressure levels.



Then the system was connected to the measurement platform. The end of one tube was placed into a vertically adjustable glass reservoir containing BSS and the end of the other tube was connected to a pressure transducer (Statham P 10 EZ, Spectramed), which was connected to a Grass recording system (Model 79 D, Grass Instrument Co.). The tube to the pressure transducer was then closed and another tube coming to the pressure transducer from a water manometer was opened. Calibration steps were drawn between 0 and 50 mmHg at increasing and decreasing pressure at 10 mmHg intervals by changing the height of the water column and using the formula $136 \text{ mmH}_2\text{O} = 10 \text{ mmHg}$. Connection to the water manometer was subsequently closed and connection between the pressure transducer and the eye was again opened. By adjusting the height of the BSS reservoir, IOP was increased for every eye from 5 mmHg to 50 mmHg and decreased back to 5 mmHg at 5 mmHg intervals in open stopcock mode. At each pressure level, IOP was allowed to stabilize and measurements were performed. Throughout the measurements, the cornea was constantly moistened with BSS. To ensure that the calibration level had not changed during the measurements, new calibration steps were drawn with water manometer after the measurements with each eye were fully performed.

2.1 Icare Measurements on Porcine Eyes

Ten enucleated porcine eyes designated as 01-10 were used to collect measurement data for the purposes of the Icare manufacturer, Tiolat Oy. Icare was carefully fastened to a vertical and horizontal position 5-6 mm away from the center of the cornea. The visual axis of the eye was aligned horizontally and all measurements at all pressure levels were performed before adjusting the eye to a vertical alignment. Ten measurements were taken at each of the 10 pressure levels at increasing and decreasing pressure and in both Icare positions. Icare was directly connected to a laptop and data were saved. Files were named as YYYYMMDD_NN_PP_O_D.txt (YYYY=year, MM=month, DD=day, NN=eye number, PP=manometer pressure in mmHg, O=Icare in vertical(X) or horizontal(Y) position, D=pressure increased(U) or decreased(D)). Results were also manually recorded to a notebook along with CCT values, measurement date and measurer name. New probe was changed at 50 mmHg, when the measurement position was changed and between eyes.

2.2 Comparison of Tonometers on Porcine Eyes

Six enucleated porcine and six enucleated bovine eyes were used to compare GAT (Nikon applanation tonometer mounted on Nikon Zoom Photo Slit Lamp Microscope FS-2), Icare (Tiolat Oy, Helsinki, Finland), Model 30 Classic™ Pneumatonometer (Medtronic Inc, Minneapolis, Minnesota, USA) and Tono-Pen XL (Mentor Inc, Norwell, Massachusetts, USA). For GAT measurements the visual axis of the eye was in horizontal alignment, for pneumatonometer in vertical alignment and for Icare and Tono-Pen XL in both horizontal and vertical alignments. Measurements were started with the eye in vertical alignment and IOP was measured by means of Icare, Tono-Pen XL and pneumatonometer at each pressure level at increasing and decreasing pressure, in that order. The eye was subsequently aligned horizontally and measurements were performed by Icare, Tono-Pen XL and GAT, in that order. Pressure was allowed to stabilize between each measurement.

2.3 Comparison of Tonometers on Bovine Eyes

Six enucleated bovine eyes were used to perform the same comparison of tonometers as with porcine eyes. The measurement protocol was also identical.

2.4 The compared tonometers in detail

2.4.1 Icare

The Icare rebound tonometer that uses the impact-induction principle(63) has been commercially available since 2003(64). It is a small-size hand-held portable tonometer with a disposable plastic probe. The probe attached to a magnetized steel wire shaft makes a momentary contact with the cornea. As the measurement button is pressed down, a magnetic field is produced in a solenoid repelling the magnetized shaft and causing the probe to hit the cornea. The probe rebounds from the surface of the cornea and induces a voltage in the solenoid(63). The induced voltage is dependent on the rebound speed of the probe. If the intraocular pressure is high, the contact time with the cornea is short and the probe bounces back faster than in lower intraocular pressures(65). Based on the induced voltage, the device calculates the IOP. After six individual measurements the device discards the lowest and highest values and shows the average of the four remaining measurements(65). A bar is shown next to the result if the standard deviation of the measurements is greater than normal(66). Due to the light weight of the probe and to the momentary contact with the cornea, the use of topical anesthetic is not required.

In this study, the device was carefully fastened in both measurement positions 5-6 mm away from the center of the cornea. One measurement was performed at each pressure level and in each position and the result was only accepted if the error bar on the LCD screen did not appear. New probe was changed between eyes and all the measurements were done by the same person.

2.4.2 Tono-Pen XL

Tono-Pen XL is a portable, hand-held device that operates on a principle similar to that of Mackay-Marg. The Tono-Pen tip has a small strain gauge located in the center of the tip. When the cornea is flattened beyond the area of the strain gauge, Mackay and Marg postulated that the forces of corneal rigidity affect mainly the edges of the tip and the only force applied to the strain gauge itself is that of IOP.(67) With each touch to the cornea, a waveform is produced, analyzed and converted to a corresponding IOP reading. In a measurement situation the tonometer tip is covered by a disposable latex cover, a drop of anesthetic is instilled in the patient's eye and measurement is performed by lightly touching the cornea. For an accepted result, four individual measurements are required and then

averaged by the device. The result is shown on the display along with a bar indicating standard deviation of either less than or equal to 5%, 10%, 20%, or >20% (68).

In the beginning of each measurement day the device was calibrated by following the instructions in the Tono-Pen XL manual. To decrease the trembling of the hand, the measurements were obtained by propping the measurement hand on the table. One measurement was performed and the result was accepted if the standard deviation shown by the device was less than or equal to 5%. All the measurements were performed by one person.

2.4.3 Pneumatonometer

IOP measurement by means of pneumatometry was first described in 1965(25). Pneumatonometers can be divided into non-contact and contact pneumatonometers. The tip of the contact pneumatonometer is a hollow tube surrounded partly by a sleeve and capped by a thin membrane. When air passes to the tube, a small gap is formed between the tip and the membrane allowing air to escape through the side exits. When placed onto the surface of the cornea, air escape is prevented because the membrane is held against the tonometer tip. Air pressure is increased until air escape again continues and the pressure in the tube balances the intraocular pressure and forces associated with corneal rigidity. The pressure sensor on the wall of the sleeve detects the air pressure and the corresponding IOP is shown on the display. Contact pneumatometry requires the use of topical anesthetic. In the non-contact pneumatonometer a short air puff applanates the cornea and a sensing device detects the mirrored light from the applanated surface. The time difference between the firing of the air puff and the detection of the light signal is the basis of IOP measurement. In low IOP the time interval from the firing to the detection is shorter than in higher IOP. Because of the noncontact nature of the measurement, no topical anesthetic is required.(29,68)

Pneumatonometer calibration was checked in the beginning of each day as instructed by the manufacturer. Two pneumatonometer measurements were obtained and the average of the values was used for analysis. Measurement hand was propped on the table to avoid trembling of the hand. The measurement was accepted if the standard deviation shown by the device was less than or equal to 0.8 mmHg and if the two measurements did not differ more than 2 mmHg. A set of two new measurements was taken until both criteria were met. One person performed all the measurements.

2.4.4 Goldmann Applanation tonometer

Invented by Goldmann and Schmidt in 1957(69), the Goldmann applanation tonometer (GAT) is the most widely used device for IOP measurements and is considered the ‘gold standard’ of tonometry. Goldmann found that the forces caused by corneal rigidity and the surface tension between the cornea and the tonometer tip are counterbalanced at an applanation diameter of 3.06 mm. With these forces canceling each other, the Imbert-Fick law describing the relationship between the pressure in a perfectly thin, elastic and dry sphere and the force required to flatten a part of this spherical surface, is valid. In addition, one gram of force applied to the corneal surface nicely corresponds to an IOP of 10 mm Hg with the Goldmann’s choice of applanation diameter. In a measurement situation GAT is mounted on to a slit-lamp microscope, in front of which the patient is asked to sit. A drop of topical anesthetic with yellow fluorescein dye is instilled in the eye, and the tonometer tip is brought in to contact of the patient’s cornea. The tonometer tip has a biprism, which splits the image in the microscope into two semicircles. By turning the measurement drum, force is applied to the cornea until applanation has been reached and a correct image of the semicircles appears in the microscope. The IOP in millimeters of mercury can be read by multiplying the drum reading by ten.(70,71)

GAT calibration was checked at a drum reading of 0, 20 and 60 mmHg by using Nikon calibration weighs. A drop of Oftan® Flurekain (Santen Inc.) containing fluorescein dye was applied to the eye before each measurement and the prism was cleaned between the measurements. Care was taken to establish semicircles that were neither too wide nor narrow. Two successive measurements were performed at each pressure level and the measurements were accepted if they were within 4 mmHg. All the measurements were done by the same person without watching the drum reading and another person recorded the results. Drum reading was reset to 0 mmHg after each measurement.

2.5 Data Analysis

Data analysis was carried out with SPSS for Windows version 11.5 and 14.5. For each eye the mean of the individual measurements at each pressure level was calculated at both increasing and decreasing pressure. The two means from the same eye and the same pressure level were then averaged and that value was used for SPSS analyses.

4 RESULTS

3.1 The Icare Measurements on Porcine Eyes

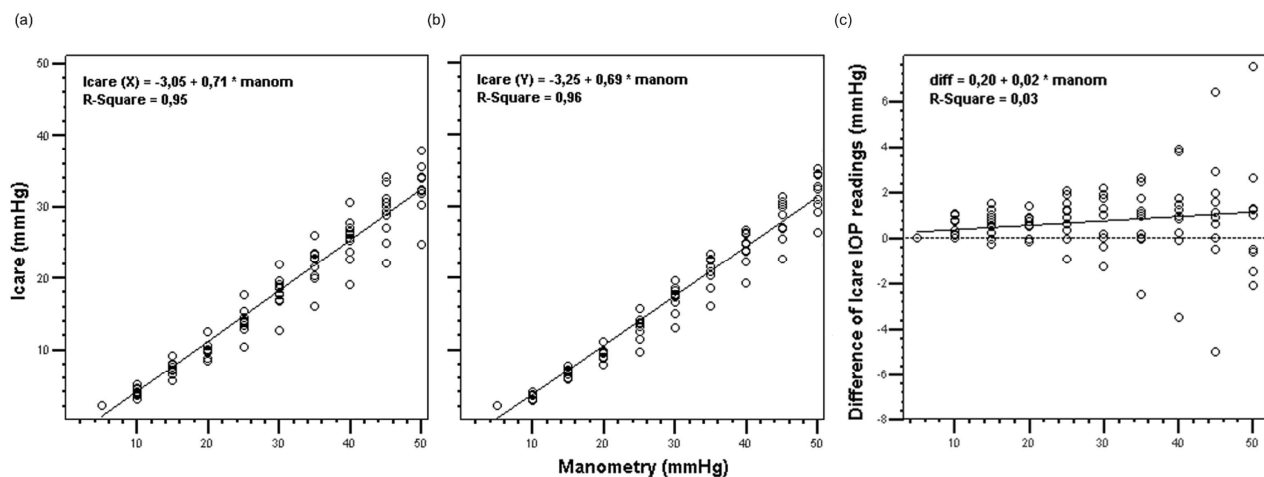
Icare consistently underestimated manometrically adjusted true IOP at every pressure level and in both measurement positions as shown in table 1. The mean IOP reading of Icare was 50 per cent or less of the true IOP at pressures ≤ 20 mmHg in both measurement positions. Although the coefficients of determination are high for the linear regression fits ($R^2=0.95$ and $R^2=0.96$ on a horizontally and vertically aligned eye, respectively (Figure 2a, 2b)), the slopes differ significantly from 1 and the constant from 0 in both measurement positions ($p<0.001$ for slope and constant in both measurement positions). When the visual axis of the eye was aligned horizontally, the mean Icare readings were slightly greater than the Icare readings on a vertically aligned eye at all pressure levels except for the 5 mmHg pressure level, where the readings were equal (Figure 2c). The variability of the between-eye measurements was also greater on horizontally aligned eyes as indicated by the standard deviations in table 1.

The mean CCT of the porcine eyes ($n=10$) measured within 8 hours after enucleation was 869.1 micrometers (standard deviation, SD 52.6). The CCT obtained just before IOP measurements was >1000 micrometers for six eyes and the mean CCT for the remaining four eyes was 960.5 micrometers (SD 42.4).

Table 1 The mean IOP reading of Icare on porcine eyes. Values are given as mean \pm standard deviation. (y = visual axis of the eye in vertical alignment, x = in horizontal alignment)

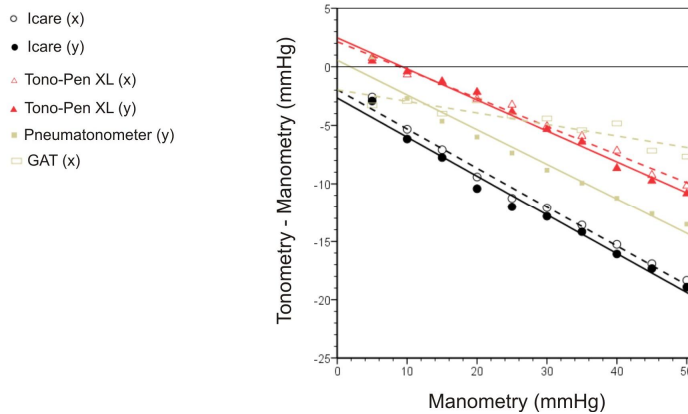
Reservoir pressure (mmHg)	Icare	
	y	x
5	2,0 \pm ,0	2,0 \pm ,0
10	3,5 \pm ,4	4,0 \pm ,6
15	6,7 \pm ,6	7,3 \pm 1,0
20	9,4 \pm ,8	9,9 \pm 1,1
25	13,1 \pm 1,6	14,0 \pm 1,9
30	17,1 \pm 1,9	17,9 \pm 2,4
35	21,0 \pm 2,2	21,7 \pm 2,6
40	24,3 \pm 2,3	25,4 \pm 3,1
45	28,1 \pm 2,7	29,1 \pm 3,7
50	32,0 \pm 2,8	33,0 \pm 3,8

Figure 2 The Icare readings of IOP on porcine eyes with the visual axis aligned horizontally (a) and vertically (b), and the difference of these two methods (c) as a function of manometric pressure setting. Lines are linear regression fits for the data. The regression equations and coefficients of determination (R-Square) are shown on the pictures.



3.2 Comparison of Tonometers on Porcine Eyes

Figure 3 The combined Altman-Bland graph comparing all tonometers with manometric pressure setting on porcine eyes. The statistical values for regression lines are: Icare (x): $R^2=0.846$, $p=0.000$ [95% limits of agreement (LA) at -21.55 to -0.82], Tono-Pen XL (x): $R^2=0.706$, $p=0.000$ [95% LA at -12.62 to 3.66], GAT (x): $R^2=0.059$, $p=0.061$ [95% LA at -16.11 to 6.82], Icare (y): $R^2=0.899$, $p=0.000$ [95% LA at -21.87 to -1.85], Tono-Pen XL (y): $R^2=0.873$, $p=0.000$ [95% LA at -12.87 to 3.23] and pneumatonometer (y): $R^2=0.945$, $p=0.000$ [95% LA at -16.22 to 0.75]. (x = visual axis of the eye in horizontal alignment, y = in vertical alignment)



Both constant and proportional bias were present in all tonometers as shown by the Bland-Altman graph (Figure 3). Icare showed the lowest accuracy of the compared tonometers in both measurement positions. All devices underestimated manometrically adjusted true IOP at pressures ≥ 10 mmHg. At 5 mmHg, Tono-Pen XL and pneumatonometer results were slightly greater than the true IOP, whereas the other tonometers underestimated the true IOP also at this pressure level. Icare readings were

slightly greater on a horizontally than vertically aligned eye. Tono-Pen XL readings on a horizontally aligned eye were slightly lower than on a vertically aligned eye at lower pressures and slightly greater at higher pressures. GAT and pneumatonometer showed the highest and lowest variability of the between-eye measurements, respectively (Table 2). The linear regression fits for all tonometers against the manometric IOP is shown in Figure 4 (in Appendix 1).

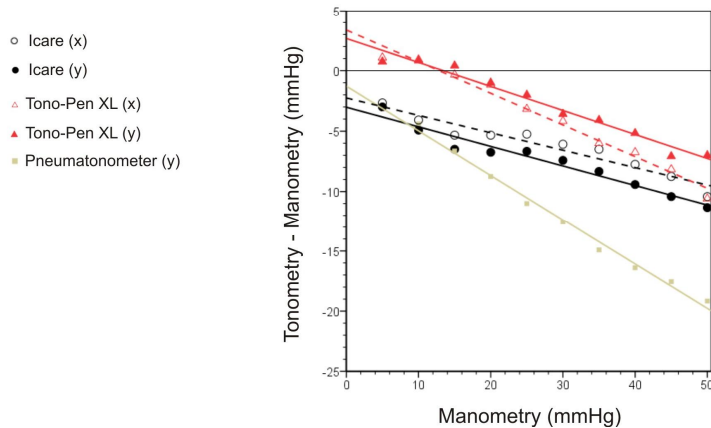
The CCT measured within 8 hours after enucleation was >1000 micrometers for two porcine eyes and the mean CCT of the four remaining porcine eyes was 925.8 micrometers (SD 37.3). The CCT obtained just before IOP measurements was >1000 micrometers for five porcine eyes and 978 micrometers for the remaining one eye.

Table 2 The mean IOP readings of Icare, Tono-Pen XL, pneumatonometer and GAT tonometers on porcine eyes. Values are given as mean \pm standard deviation. (y = visual axis of the eye in vertical alignment, x = in horizontal alignment)

Reservoir Pressure (mmHg)	Icare		Tono-Pen XL		Pneumatonometer	GAT
	y	x	y	x	y	x
5	2,1 \pm ,2	2,4 \pm ,5	5,5 \pm ,8	5,8 \pm ,7	5,9 \pm ,4	1,7 \pm 1,7
10	3,8 \pm ,7	4,7 \pm ,5	9,6 \pm ,4	9,3 \pm ,4	7,3 \pm ,5	7,1 \pm 2,5
15	7,3 \pm ,4	7,9 \pm ,7	13,8 \pm ,7	13,7 \pm 1,1	10,4 \pm ,5	11,0 \pm 3,3
20	9,6 \pm ,4	10,6 \pm 1,2	17,8 \pm 1,2	17,3 \pm 1,9	14,0 \pm ,8	17,1 \pm 3,9
25	13,0 \pm ,8	13,8 \pm 1,6	21,2 \pm 1,5	21,8 \pm 2,7	17,6 \pm ,7	20,8 \pm 4,9
30	17,2 \pm 1,4	17,9 \pm 2,2	24,7 \pm 2,0	24,9 \pm 2,4	21,2 \pm ,6	25,6 \pm 6,7
35	20,8 \pm 1,4	21,4 \pm 2,2	28,6 \pm 1,7	29,1 \pm 3,2	25,1 \pm ,8	29,6 \pm 4,7
40	23,9 \pm 1,9	24,8 \pm 2,5	31,3 \pm 1,6	32,8 \pm 2,8	28,8 \pm ,8	35,2 \pm 8,5
45	27,7 \pm 2,5	28,1 \pm 3,3	35,3 \pm 2,3	35,8 \pm 3,1	32,4 \pm 1,1	37,8 \pm 9,7
50	31,1 \pm 3,2	31,7 \pm 3,9	39,2 \pm 2,0	39,8 \pm 3,4	36,5 \pm 1,1	42,3 \pm 9,0

3.3 Comparison of Tonometers on Bovine Eyes

Figure 5 The combined Altman-Bland graph comparing all tonometers with manometric pressure setting on bovine eyes. The statistical values for regression lines are: Icare (x): $R^2=0.511$, $p=0.000$ [95% limits of agreement (LA) at -11.94 to -0.50], Tono-Pen XL (x): $R^2=0.753$, $p=0.000$ [95% LA at -12.41 to 4.74], Icare (y): $R^2=0.660$, $p=0.000$ [95% LA at -13.13 to -1.82], Tono-Pen XL (y): $R^2=0.439$, $p=0.000$ [95% LA at -11.31 to 5.72], pneumatonometer (y): $R^2=0.782$, $p=0.000$ [95% LA at -23.05 to -1.67]. (x = visual axis of the eye in horizontal alignment, y = in vertical alignment)



Pneumatonometer was the least accurate and Tono-Pen XL the most accurate tonometer of the compared devices on bovine eyes (Figure 5). Icare and pneumatonometer underestimated true IOP at all pressure levels whereas Tono-Pen XL overestimated at lower pressures and underestimated at higher pressures. Icare and Tono-Pen XL readings were higher and pneumatonometer readings lower on bovine than on porcine eyes. As with porcine eyes, Icare readings were slightly

greater on a horizontally than vertically aligned bovine eye. Tono-Pen XL readings on a horizontally aligned bovine eye were slightly greater than on a vertically aligned eye at lower pressures and slightly lower at higher pressures. Tono-Pen XL and Icare on a vertically aligned eye showed the highest and lowest variability of the between-eye measurements, respectively (Table 3). The linear regression fits for all tonometers against the manometric IOP is shown in Figure 6 (in Appendix 1).

The CCT of all six bovine eyes was >1000 micrometers.

Table 3 The mean IOP readings of Icare, Tono-Pen XL and pneumatonometer on bovine eyes. Values are given as mean \pm standard deviation. (y = visual axis of the eye in vertical alignment, x = in horizontal alignment)

Reservoir pressure (mmHg)	Icare		Tono-Pen XL		Pneumatonometer
	y	x	y	x	y
5	2,0 \pm ,0	2,3 \pm ,4	5,8 \pm 1,4	6,1 \pm ,7	. .*
10	5,1 \pm ,6	5,9 \pm 1,0	10,8 \pm 1,3	10,9 \pm 1,1	5,6 \pm ,4
15	8,5 \pm 1,0	9,7 \pm 1,1	15,4 \pm 1,7	14,7 \pm 1,3	8,4 \pm 1,8
20	13,3 \pm 1,4	14,7 \pm 1,8	19,0 \pm 1,5	18,8 \pm 2,0	11,3 \pm 1,9
25	18,3 \pm 1,6	19,8 \pm 2,1	23,0 \pm 2,4	21,8 \pm 2,1	14,0 \pm 2,0
30	22,6 \pm 2,0	23,9 \pm 2,2	26,4 \pm 3,5	25,8 \pm 1,9	17,4 \pm 2,2
35	26,7 \pm 2,2	28,5 \pm 2,6	30,9 \pm 4,1	29,0 \pm 2,1	20,1 \pm 3,1
40	30,6 \pm 2,0	32,3 \pm 2,7	34,8 \pm 3,1	33,3 \pm 1,8	23,6 \pm 3,2
45	34,6 \pm 2,0	36,3 \pm 2,8	37,9 \pm 4,7	36,8 \pm 2,9	27,5 \pm 4,2
50	38,7 \pm 2,7	39,6 \pm 2,9	43,0 \pm 6,8	39,4 \pm 4,6	30,8 \pm 3,7

*) Only one successful measurement result (5,5 mmHg) was obtained with pneumatonometer from the reservoir pressure of 5 mmHg.

5 DISCUSSION

The aim of this study was to evaluate the feasibility of an experimental *in vitro* IOP model for the development of a continuous IOP measurement device. The feasibility was evaluated by comparing the IOP measurement results with four tonometers against the true IOP.

The study showed that manometrically adjusted IOP on enucleated porcine eyes can be measured with GAT, pneumatonometer, Tono-Pen XL and Icare. On bovine eyes IOP measurements can be performed only with pneumatonometer, Tono-Pen XL and Icare. As the correct image of the semicircles was reached without applying any force at the 50 mmHg pressure level, IOP measurement with GAT is not possible on bovine eyes with the standard tonometer tip. Even though the bovine eye properties other than the CCT were not measured in this study, it is obvious that the bovine cornea is substantially bigger in diameter and thus flatter than that of human or porcine eye. Ubels et al calculated in their study the bovine corneal radius of curvature to be 17.4 and 13.1 mm in the horizontal and vertical axes, respectively, which is roughly twice that of human cornea(72). In our study the CCT of bovine cornea was over 1 mm within 12 hours of enucleation whereas Doughty measured in his study the CCT of bovine cornea to be about 1007 micrometers one hour after enucleation and 1218 micrometers after 24-hour storage in +4 degrees Celsius(73). In addition to being structurally thicker than the human cornea, the bovine cornea was also edematous in this study. Since structurally thick cornea should overestimate GAT readings(25) it is probably due to the large radius of curvature and corneal edema why GAT measurement failed on bovine eyes. In addition, there were considerable difficulties with pneumatonometer on bovine eyes at the lower pressures and we were only capable of getting one measurement result from 5 mmHg pressure level. Such difficulties were not perceived with porcine eyes.

The accuracy of tonometers on both porcine and bovine eyes was limited as all tonometers considerably underestimated the true IOP. In addition to a constant bias, there was a bigger underestimation at higher pressures because proportional bias was also present. The analysis showed a linear correlation between manometric IOP and error in Icare, Tono-Pen XL and pneumatonometer. The linear correlation between manometric IOP and error in GAT was not significant ($p=0.061$). Either

there is no linear correlation because the error in GAT is constant and not dependent on the adjusted IOP level (only constant bias present) or there was too large proportion of variability between measurements. The variability as judged by the standard deviation is considerably bigger than with other tonometers as seen in table 2. The maximum standard deviation was 9.7 mmHg with GAT, whereas with Icare, Tono-Pen XL and pneumatonometer on porcine eyes the maximum standard deviations were 3.9, 3.4 and 1.1 mmHg, respectively. The large variability with GAT can be attributed mainly to two things: First, the measurer was not entirely experienced with the use of GAT. Secondly, GAT measurement seems to be significantly influenced by highly edematous corneas as noted in another study by Kniestedt et al(74), why the judgment of correct semicircle position was occasionally challenging. Because GAT measurements on enucleated porcine eyes have been possible with lower variability in other studies, it is most likely due to the inexperience of the measurer why standard deviations were large. However, when the tonometric IOP was plotted against the manometric IOP, the linear regression model was significant at the $p < 0.001$ level also for GAT (Figures 4 & 6).

On enucleated porcine eyes Hallberg et al(75) reported that GAT underestimated the true IOP on average 14 mmHg between IOP range of 10 and 40 mmHg. In this study the average underestimation between pressure range of 5 and 50 mmHg was 12, 11, 5, 4, 7 and 5 mmHg for Icare (visual axis vertically), Icare (visual axis horizontally), Tono-Pen XL (visual axis vertically), Tono-Pen XL (visual axis horizontally), pneumatonometer and GAT, respectively. On enucleated bovine eyes the average underestimation was 7, 6, 3, 4 and 12 mmHg for Icare (visual axis vertically), Icare (visual axis horizontally), Tono-Pen XL (visual axis vertically), Tono-Pen XL (visual axis horizontally) and pneumatonometer. Except for pneumatonometer, the average underestimation was less on bovine than porcine eyes.

Substantial corneal edema was present both in bovine and porcine corneas. Corneas were thicker at the time of IOP measurement than at the time when the CCT was measured the first time after enucleation. The delay between the enucleation and IOP measurement was 8 hours at minimum and 5 days at maximum. A study reported the normal porcine cornea to be approximately 780 micrometers thick and thickening 26 micrometers per hour after enucleation(76). Still another study measured the CCT of enucleated porcine cornea to be on average 842 micrometers(75) and another study 850 micrometers(77). Corneal edema is associated with underestimation of IOP by tonometry, which might

be one factor explaining the results in this study(25). For example, one study found that GAT and pneumatonometer underestimated the manometrically adjusted IOP more in hydrated than dehydrated enucleated human corneas(74).

Although not measured in this study, the corneal radius of curvature of porcine cornea is measured at approximately 8.9mm(75). In normal human cornea the radius of curvature is 7.8mm, which might partly explain, at least for GAT, the underestimation in our results. Bigger corneal radius of curvature and thus flatter cornea allows the full contact area of the tonometer tip to be reached with less force. The adhesion force of the corneal fluid also pulls the tip towards the eye with more force than in an eye with smaller cornea radius of curvature(75).

In further studies, the delay between enucleation and IOP measurement should be minimized in order to avoid the influence of corneal edema on the results. Alternatively, a hypertonic solution, such as 20% dextran, could be introduced into the anterior chamber and the globe could be stored in such a solution if not used in the same day to dehydrate the edematous cornea.

IOP can be adjusted in an experimental model either by closed or open stop-cock technique. In this study, the open stop-cock method was used because it allowed us to keep the pressure at a constant level during the measurements. In the closed stop-cock method, the IOP increases as external pressure, such as measurement probe, is introduced on the surface of the cornea. Although the closed method is similar to the *in vivo* conditions, after the initial pressure increase the IOP will begin to decline because of leakage of fluid from the enucleated eye. In a study by Eisenberg et al(78) there was almost a 50% reduction in the pressure three minutes after closing the stop-cock. Kniestedt et al(79) suggested that it is difficult to decide at which timepoint to record the manometer pressure in a closed stop-cock system. For practical purposes, therefore, it might be more convenient to continue to use the open stop-cock method also in further studies.

Regardless of the gross underestimation of the tonometric IOP, the relationship between all tonometers and manometric IOP was linear and the linear regression equations were significant for all tonometers. The underestimation can be taken into account by a calibration algorithm which can be calculated from this linear relationship. The aim in the development of a continuous IOP monitoring device is to find a

relationship between the measured IOP and the manometric IOP and to calculate a calibration algorithm based upon this relationship. As the relationship was found to be linear with currently available tonometers, all of which measured the IOP outside the cornea, it is probable that the pressure sensor implanted intracorneally can also track the changes in manometric IOP. In conclusion, the experimental *in vitro* IOP model used in this study is deemed feasible for the development of a continuous IOP monitoring device.

APPENDIX 1

Figure 4 The tonometric IOPs plotted against the manometric pressure setting on porcine eyes. Lines are linear regression fits for the data. The regression equations and coefficients of determination are shown on the pictures. The linear regression model significantly fits the data at the $p < 0.001$ level with every tonometer (a-f). (x = visual axis of the eye in horizontal alignment, y = in vertical alignment)

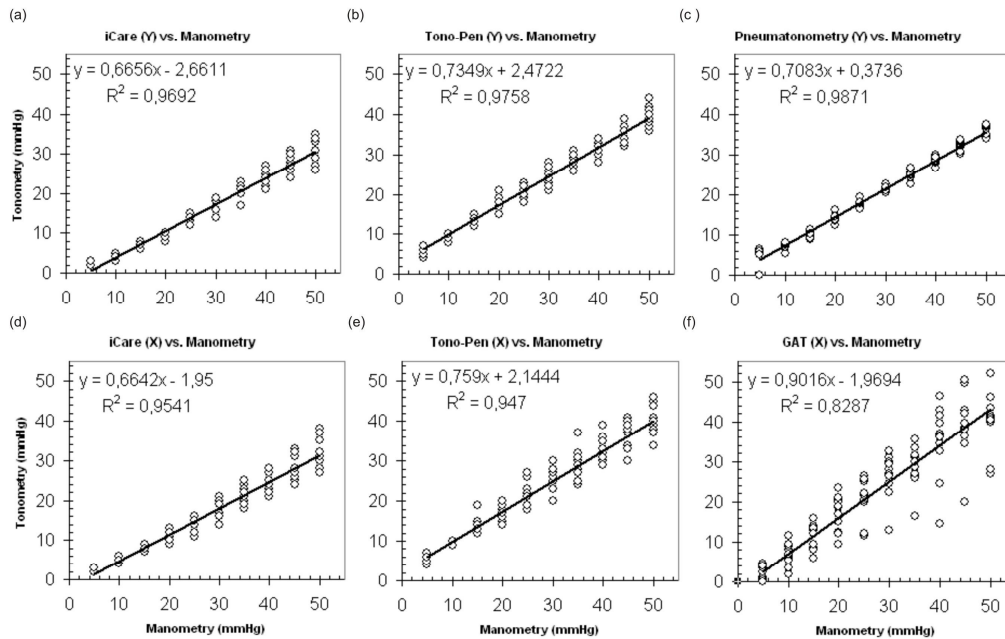
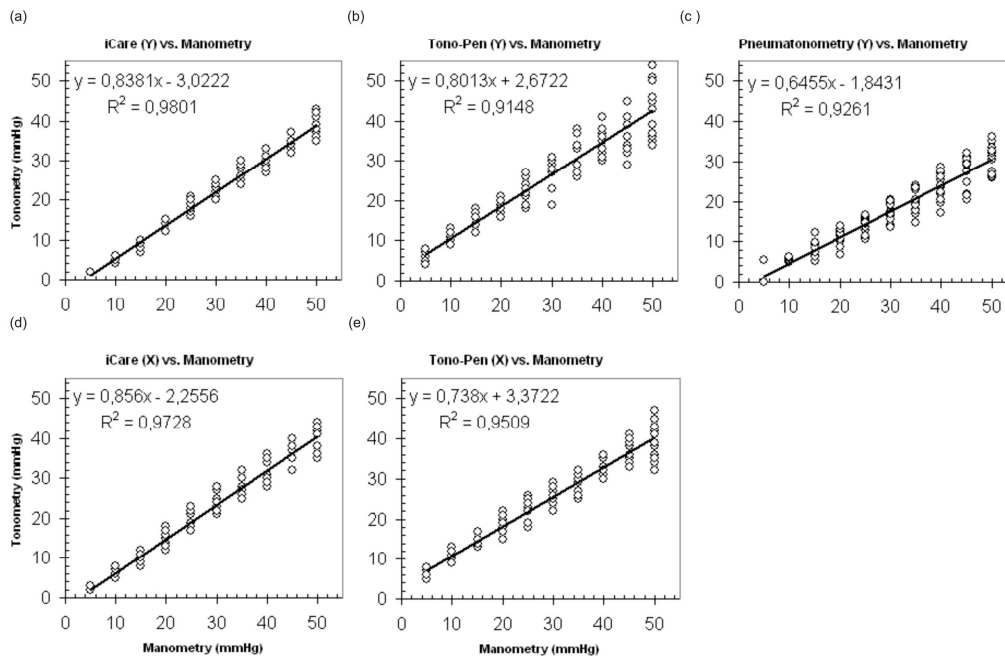


Figure 6 The tonometric IOPs plotted against the manometric pressure setting on bovine eyes. IOP measurement with GAT could not be performed on bovine eyes. Lines are linear regression fits for the data. The regression equations and coefficients of determination are shown on the pictures. The linear regression model significantly fits the data at the $p < 0.001$ level with every tonometer (a-e). (x = visual axis of the eye in horizontal alignment, y = in vertical alignment)



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